1490-Pos Board B260
The Threshold Force for Membrane Tether Formation Depends Strongly on Loading Rate
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Tethers are thin tubes of lipids (~20-200 nm in diameter) that form when membranes are subjected to a point force. Tether dynamics are important to a myriad of biological processes including white blood cell adhesion and transport of intracellular material between neighboring cells. To understand the dynamics of tether formation more fully, we investigated the dependence of the force needed to create a tether on the rate of force change (loading rate). To conduct these experiments, a microfabricated magnetic force transducer was used to generate well-controlled and localized magnetic force profiles. Tethers were formed off the surface of microaspirated giant unilamellar vesicles (GUVs) attached to magnetic beads. We discovered a strong correlation between the threshold force of tether formation and the applied force ramp, with the force changing from <10 pN at low loading rates to ~50 pN at high loading rates. At slow loading rates, the threshold force changes weakly with ln (loading rate), while at high loading rates a steeper dependence is observed. The experimental data can be fit to a energetic model based on Kramer's theory, similar to models used to describe membrane rupture. The model predits that tether formation involves passage over two energy barriers and enbales characterization of the characteristic forces and timescales associated with these barriers. This new tool for dynamic studies of membrane mechanics may further be extended to study how tethers form off of flowing cells or how phase regimes, induced by the presence of cholesterol, influence membrane dynamics.

1491-Pos Board B261
Solid-State ^{2}H NMR Reveals Changes in Membrane Flexibility Due to Osmotic Pressure
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Cellular membrane properties are sensitive to pressure, temperature, and dehydration as well as lipid composition, which can affect function through non-specific lipid-protein interactions [1]. Functional lipid rafts in cellular membranes may correspond to detergent-resistant domains due to the presence of cholesterol. Changes in swelling and stiffening of pure lipid bilayers in the liquid-crystalline phase have been observed [2-4] with addition of detergent and cholesterol. Here we show how structure and associated dynamics of mixed-lipid bilayers are affected by osmotic pressure. Determinations of area per lipid and motional parameters of DMPC membranes in the presence of detergent ($C_{12}E_8$) or cholesterol utilize ^{2}H NMR together with a mean-torque model for interpreting acyl-chain order parameters (S_{CD}) [5]. Swelling by addition of detergent is due to enhanced membrane flexibility, and is counteracted by applying osmotic pressure to the lipid dispersion. By contrast, reduced swelling of multilamellar dispersions due to the stiffening action of cholesterol is reinforced by osmotic pressure. In both cases the membrane area compressibility modulus K_a is calculated from S_{CD} order parameters. We propose that apparent K_a values differ with osmotic pressure for both systems due to changes in the hierarchy of forces and motions. Calculation of the bilayer bending rigidity and the area elastic modulus provides a basis for molecular dynamics simulations of membrane deformations at the atomistic and mesoscopic levels. Osmotic pressure-induced deformation of membranes reveals how lipid-protein interactions can play key roles in biological functions of pressure-sensitive proteins and channels. [1] K.J. Mallikarjunaiah *et al*. (2011) *BJ* **100**, 98-107 [2] M.F. Brown *et al*. (2002) *JACS* **124**, 8471-8484. [3] D. Otten *et al*. (2000) *JPC* **104**, 12119-12129. [4] G.V. Martinez *et al*. (2002) *PRE* **66**, 050902. [5] H.I. Petrache *et al*. (2000) *BJ* **79**, 3172-3192.

1492-Pos Board B262
Inhibition of the Peroxidation of Liposomal Lipids by Uric Acid Requires Tocopherol
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Urate is the major water-soluble low molecular weight antioxidants in serum, contributing about 50% to the antioxidative potential of the serum. Unexpectedly, both urate, as well as the other major antioxidant ascorbate, promote the copper-induced peroxidation of liposomal PUFA. In a previous study it has been shown that ascorbate inhibits copper-induced oxidation of liposomal lipids when the liposomes contain Tocopherol, whereas urate does not. In an attempt to explain these findings we studied the temporal order of events, by monitoring continuously and simultaneously the time-course of formation of oxidation products and the consumption of the various components of the system. The resultant kinetic profiles show that: 1. Both water-soluble antioxidants slightly inhibit the oxidation of tocopherol; 2. Ascorbate becomes oxidized very rapidly (much faster than tocopherol), whereas urate and tocopherol become oxidized simultaneously. 3. In the presence of tocopherol, both urate and ascorbate inhibit copper-induced peroxidation of PUFA. 4. AAPH-induced peroxidation of liposomal PUFA is inhibited by both urate and ascorbate, independent of the presence of tocopherol. Our interpretation of these results contribute to the understanding of the complex, interdependent dependence of the susceptibility of aggregated (unlike soluble) lipids on all the water-soluble antioxidants. This is particularly important for evaluation of the oxidizability of serum lipids when the serum contains excess urate, as in the case of insulin-resistant and obese subjects.

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From Thermodynamic States to Biological Function by Einstein's Approach to Statistical Physics
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Einstein founded statistical physics on an important generalization of the Boltzmann principle: Einstein's reversion, where not the model but the law of entropy is placed first. The advantage is that it assures the 2nd Law and requires no model assumptions. In particular, the existence of a complete molecular mechanism is not necessary. However, if the empirical behavior of a system is known, the entropy and the corresponding probability of the thermodynamic states can be directly derived. With his approach Einstein successfully explained Brownian motion, wave-particle dualism, quantum transitions as well as the Bose-Einstein condensation.
Impossible to find in any textbook, we outline Einstein's approach and apply it to soft interfaces. The introduction of their proper entropy potential, its first, and its second derivatives predicts interfacial nonequilibrium excitation, propagation, and fluctuations, respectively. Experimental observations of the phenomenology of the membrane susceptibilities allow quantitative predictions. The propagation of waves as well as the existence of channel-like current fluctuations are experimentally confirmed and compared to measurements on living systems.
Finally, we present experiments that confirm Einstein's approach to the interfacial reaction coordinate. Here the phenomenology of the system is derived from a proper entropy law even though hidden from direct observation. Not structure of molecules but entropy of the aqueous interfaces turns out to be the origin of catalysis and the associated surprising increase in reaction rate. Simultaneous specificity and activity appears now predictable and no more paradox. The theory derived from K.K. in 1999 is briefly outlined and confirmed in experiments on Acetylcholinesterases incorporated in lipid monolayers. Since enzyme activity is controlled from remote by these continuous layers, our results predict the ubiquitous, integrative action in biology of the excitable hydration interface.

1494-Pos Board B264
Polymer Mediated Interactions Between Myelin Lipid Bilayers
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The axons of the central nervous system are covered by a multi-layered membrane which provides insulation and high electric signal conductivity to the axons. Swelling of the myelin sheaths is the hallmark of many neurological degenerative diseases like multiple sclerosis. Loss of the myelin membrane involves subtle changes in the interaction forces that hold the membrane stack together. These interaction forces are believed to have more than one origin, the major one arising from the dedicated protein -myelin basic protein (MBP) - which binds to, and bridges, the cytoplasmic sides of myelin membrane via electrostatic and hydrophobic interactions. It has been shown that deimination of MBP leads to a loss of adhesion between myelin membranes and ultimately to swelling of myelin. In the present study, we explored a new strategy that makes use of structured polymers to reverse the effects of loss of adhesion between myelin membranes. By controlling the chemical composition and architecture of the polymers, our results show that it is possible to enhance the adhesion between the membranes using different types of interaction forces like electrostatic forces, depletion forces, bridging forces or combinations of them. Neutral triblock copolymers with a central hydrophobic segment present a strong affinity to the bilayers and enhance the adhesive interaction forces between the membranes via a depletion mechanism similar to hydrophilic homopolymers (like PEG). On the other hand, triblocks copolymers with two

hydrophobic lateral blocks promote adhesion between bilayers via bridging interaction controlled by the concentration and segments chain length of the polymer. The results pave a new route to the development of treatments for this debilitating disease and shed a new light on the relationship between polymer structure, self assembly and interactions with complex biomembranes.

Membrane Structure I

1495-Pos Board B265

Temperature Behavior of Nanometer-Size Lipid Domains in DPPC:DLPC Model Membranes Studied by Small Angle Neutron Scattering

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Cellular membranes are no longer viewed as a heterogeneous mix of lipids and proteins, but rather as having distinct lipid domains which are key for many biological processes. Much work devoted to understanding the mechanisms that drives lateral organization in cell membranes has been done on model membrane systems. The study of lipid-lipid phase separation contributes to our understanding of the structure-function relation in the cell membrane. Phase behavior of lipid mixtures has been studied extensively in model lipid membranes using microscopy and spectroscopy techniques. By microscopy, the domains are found to be microns in size. A hypothesis to explain small domains in real cell membranes is that the cytoskeleton generates boundaries, generating small membrane regions with access to only a small amount of lipids and other components. Therefore, to be able to correlate studies of model membranes to the actual plasma membrane, there is a need to characterize lipids domains in a system where they cannot grow more than few nanometers in size. To achieve such a goal, we use Small Unilamellar Vesicles made of a 1:1 ratio of deuterated DPPC and DLPC for which phase separation in large vesicles has been observed. Small Angle Neutron Scattering was used to characterize the size and composition of the domains, which appeared as the temperature was lowered below the Tm of the system (which lies between the two Tm values of each lipid depending on the composition). The scattering was fitted using an ab initio method developed by Svergun and colleagues to analyze scattering data from biological macromolecules. The results show that domains in these systems do not coalesce to form a single stable domain but rather break-up into smaller domains as the temperature lowers.

1496-Pos Board B266

Physical Properties of Lipid Membranes Containing Sterol Studied by Deuterium NMR and Fluorescence Microscopy

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We study the physical properties of model membranes composed of phosphatidylcholines and sterol. The morphology and phase behavior of membranes were investigated by deuterium NMR and fluorescence microscopy. In binary mixtures, coexistence of solid-ordered and liquid-disordered phases was observed in a wide temperature and composition range. The results for ternary mixtures containing sterol show that addition of sterol promotes the formation of the liquid-ordered phase. Sterol has strong influence on the morphology and the phase behavior of membranes. A partial phase diagram of will be presented.

1497-Pos Board B267

Role of Curvature to Produce Modulated Patterns of Lo + Ld Phases on GUV Surfaces

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GUV studies of the three component lipid system DSPC/DOPC/CHOL show macroscopic phase separation of Ld + Lo phase domains. In contrast, GUVs of DSPC/POPC/CHOL appear uniform in the Ld + Lo region, but show nanometer-scale phase separation in FRET and ESR studies. Examination of the four component system DSPC/DOPC/POPC/CHOL enables study of macroscopic-to-nanoscopic transition as composition (DOPC/POPC ratio) is changed. The transition is observed to be rather abrupt, and reveals ''modulated phase'' patterning on the GUV in a small window of compositions. The patterns show stripe, bubble, and honeycomb structures. Following Helfrich, we attempt to explain these patterns through the energetics of curvature. We perform calculations and simulations to determine whether a heterogeneous membrane can stabilize modulated phases, subject only to a line tension and a bending rigidity. We also explore the energetic stability, shape, and size scales of these patterns.

1498-Pos Board B268

Cholesterol Mediates Membrane Curvature during Fusion Events

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Biomembranes undergo extensive shape changes as they perform vital cellular functions. The molecular mechanisms by which lipids and proteins generate and control membrane curvature remain largely unclear. Membrane curvature can be generated by a protein that embeds its amphipathic domains into the lipid matrix. Shallowly embedded domains expand, mainly, the lipid polar heads, while the hydrocarbon chains remain undisturbed. This leads to a strong asymmetry in spacing between the tails and headgroups of the membrane lipids and, consequently, to generation of a positive membrane curvature. In contrast, when a protein's domain expands the polar and hydrocarbon regions of the membrane lipids evenly, only negligible curvature is produced. We present evidence that cholesterol could directly control the embedding depth and occupied surface area of protein's amphipathic domains, and thus induced membrane curvature on the example of fusion between HIV-1 viral envelope and host cell membranes. Bending of the host cell membrane in this process is mediated by the N-terminal fusion domain of the viral glycoprotein gp41. DPPC/cholesterol monolayers at the air/liquid interface with different ratios of cholesterol were used to mimic the host cell membrane. The membrane binding properties of the fusion domain were assessed with the constant-pressure insertion assays and X-ray reflectivity. When the concentration of cholesterol is low, the gp41 protein's domain embeds shallowly into the lipid matrix, causing significant surface area perturbations and generating positive membrane curvature. In contrast, deep insertion of the fusion domain implies that it produces negligible curvature in membranes with high cholesterol content. Taken together, previous reports and our data offer a new mechanism of how lipids and proteins could regulate membrane curvature - in response to the protein domains' binding cholesterol may rearrange locally thereby altering membrane-binding properties of these domains and curvature they produce.

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Calculating Elastic Constants and the Effects of Curvature on the Binding of Lipid Chain Anchors to DPPC/DOPC/Cholesterol Model Lipid Bilayers

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Using a theoretical model of a bilayer membrane containing cholesterol, dipalmitoyl-phophatidylcholine (DPPC), and dioleoylphosphatidylcholine (DOPC) that qualitatively reproduces phase diagrams of giant unilaminar vesicles (GUVs) of the same three components [R. Elliott, I. Szleifer, and M. Schick, Phys. Rev. Lett., 96, p.098101 (2006)], we calculate the bending and saddle-splay force constants in Gel, liquid-ordered (lo), and liquid-disordered (ld) phases. The molecular theory employed in our study allows us to determine the effects of the mode of membrane bending deformation on the value of the elastic constants for different phases. The effect of ''blocked'' vs ''free'' exchange of lipids across the two monolayers on the values of the bending constant is as high as 50 kbT in the ld phase to as high as 200 kbT in the lo phase. These results show that one must strongly consider the mode of deformation in regard to the mechanical properties of lipid bilayers. For example, if cholesterol is allowed to flip-flop and the other lipid species are ''blocked'', then the bending elastic constant is 20-40 kbT larger then the case where all of the lipid species are allowed to be exchanged from leaflet to leaflet.
We will also present results on how the curvature of lipid vesicles determines the amount of binding of molecules with lipid tail anchors. By explicitly determining the chemical potential difference of species across a curved bilayer under different modes of deformation, we are able to calculate the equilibrium binding concentrations of lipid tail anchors as a function of membrane curvature, concentration of lipids, and local electrostatic environment.

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Cholesterol-Lipid Affinity Determined by EPR

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Cholesterol-lipid interactions are thought to play an intrinsic role in determining lateral organization within cellular membranes. Rafts enriched in cholesterol are ''glued'' together by the high affinity that the sterol has for sphingolipids, whereas poor affinity for cholesterol is hypothesized to drive the formation of polyunsaturated fatty acid (PUFA)-containing phospholipid domains depleted in the sterol. Here we describe a novel method using electron paramagnetic resonance (EPR) to measure the affinity of cholesterol for model membranes. Our method determines the relative amount of cholesterol that partitions between large unilamellar vesicles (LUV) and cyclodextrin by analyzing